# High-Efficiency, Selection-free Gene Repair in Airway Stem Cells from Cystic Fibrosis Patients Rescues CFTR Function in Differentiated Epithelia.

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**Public Summary:**
Cystic fibrosis (CF) is a genetic disease caused by mutations in a gene called CFTR that affects many organ systems, but the most important organ for the survival of patients is the respiratory system. 70% of patients have the same mutation that causes CF called "df508." We have developed a gene editing system that efficiently corrects this mutation in the airway stem cells from patients. The correction efficiency is high enough that it restores function to airway sheets derived from the genetically corrected airway stem cells. Moreover, we demonstrate that the cells corrected outside the body can be integrated into a biomaterial scaffold that has been frequently used in patients for other purposes. In the future, we propose to determine how to transplant the cell embedded scaffold into animals and one day patients such that the patient's own genetically corrected airway stem cells can recreate the lining of the airway with a functioning CFTR and thereby successfully treat the airway problems for patients with CF.

**Scientific Abstract:**
Cystic fibrosis (CF) is a monogenic disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Mortality in CF patients is mostly due to respiratory sequelae. Challenges with gene delivery have limited attempts to treat CF using in vivo gene therapy, and low correction levels have hindered ex vivo gene therapy efforts. We have used Cas9 and adeno-associated virus 6 to correct the DeltaF508 mutation in readily accessible upper-airway basal stem cells (UABCs) obtained from CF patients. On average, we achieved 30%-50% allelic correction in UABCs and bronchial epithelial cells (HBECs) from 10 CF patients and observed 20%-50% CFTR function relative to non-CF controls in differentiated epithelia. Furthermore, we successfully embedded the corrected UABCs on an FDA-approved porcine small intestinal submucosal membrane (pSIS), and they retained differentiation capacity. This study supports further development of genetically corrected autologous airway stem cell transplant as a treatment for CF.